Secretion of a Cytoplasmic Lectin from *Xenopus Laevis Skin*

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Abstract. The skin of *Xenopus laevis* contains a soluble β-galactoside-binding lectin with a ~16,000-mol-wt subunit. It resembles similar lectins purified from a variety of tissues from other vertebrates, and differs from two other soluble *X. laevis* lectins from oocytes and serum that bind α-galactosides. The skin lectin is concentrated in the cytoplasm of granular gland and mucous gland cells, as demonstrated by immunohistochemistry with the electron microscope. Upon injection with epinephrine, there is massive secretion of the cytoplasmic lectin from the granular gland cells.

SOLUBLE lectins are widely distributed in vertebrates (1). Immunohistochemical studies of their distribution generally demonstrate both intracellular and extracellular lectin in a given tissue (1). These and other studies suggest that the intracellular lectin may function only upon secretion to an extracellular site, where it could associate with complementary glycoconjugates on the cell surface or in the extracellular matrix (1).

Secretion of soluble vertebrate lectins has been demonstrated directly only in the case of chicken lactose lectin II (3). It is localized along with mucin in the secretory vesicles of the goblet cells of the intestinal mucosa, and both are secreted upon cholinergic stimulation (3). In contrast, other soluble vertebrate lectins are found in the cytoplasm, not in vesicles, making it hard to accept that they are truly secretory proteins. For example, the intracellular localization of a rat β-galactoside-binding lectin, which accumulates extracellularly in the elastic fibers of lung, is cytoplasmic, not vesicular (8). Yet it must be secreted, since it is ultimately concentrated in extracellular matrix.

In the course of studies of the distribution of α-galactoside-binding of soluble lectins in *Xenopus laevis* (24, 25) we found β-galactoside-binding lectin activity in skin extracts, previously detected by Nitta et al. (19). Since skin is a major secretory organ in *Xenopus* (14), this provided a potential opportunity to study lectin secretion. In the present report we describe purification and immunohistochemical localization of a β-galactoside-binding lectin from *Xenopus* skin. The lectin is highly concentrated in the cytoplasm of the granular gland, from which it is secreted upon systemic injection with epinephrine. The results provide a dramatic example of secretion of a cytoplasmic lectin, without packaging in vesicles.

Materials and Methods

Lectin Purification

Skin was obtained from female *Xenopus laevis*, purchased from Xenopus I (Ann Arbor, MI). Animals were killed and 7–10 g of skin were obtained from the trunk of a single specimen. Dissections and subsequent procedures were done at 4°C.

The skin was rinsed in saline and cut into small pieces with a razor blade. The pieces were mixed with 10 ml of 50 mM Tris-HCl, pH 7.6, that contained 0.15 M lactose, 10 mM EDTA, and 4 mM β-mercaptoethanol, and frozen in liquid nitrogen. The sample was thawed, homogenized in a Sorvall Omni mixer for 1 min at setting 5, then frozen in liquid nitrogen and again thawed. The above buffer was added to the homogenate to give 9 ml of buffer for each gram of skin, and this more dilute homogenate was again homogenized in a Sorvall Omni mixer at setting 5, but for 10 min. The final homogenate was passed through three layers of cheese cloth and centrifuged for 1 h at 100,000 *g*. The supernatant was dialyzed in tubing with a molecular weight cutoff of 8,000 against 50 mM Tris-HCl, pH 7.6, that contained 10 mM EDTA and 4 mM β-mercaptoethanol (TEM).[1] This solution was changed frequently so that ultimately dialysis occurred against ~20 liters of buffer. During the course of dialysis, a precipitate began to accumulate, and at the end of dialysis this was removed by centrifugation at 100,000 *g* for 1 h. The supernatant was used for affinity chromatography.

Affinity chromatography was performed on 2-ml columns of agarose gel beads conjugated with lactose (Lactose II, Pierce Chemical Co., Rockford, IL). An extract from a single animal contained from 100 to 250 mg of protein and was applied to a column that had been equilibrated with TEM. The solution that passed through was collected and passed over the column again. Afterwards, the column was washed with 80 ml of TEM, 25 ml of 0.3 sucrose in TEM, and again with 80 ml of TEM. The lactose-binding lectin was eluted with 4 ml of 0.3 M lactose in TEM.

Further purification was achieved by DEAE ion exchange chromatography on a Bio-Rad TSK 5 PW column using a Perkin Elmer Series 4 liquid chromatograph equipped with a LC-95 variable wavelength detector and LCI-100 recording integrator. The material eluted from the affinity column was first concentrated by ultrafiltration and dialyzed against 10 mM NaCl, 10 mM Tris-HCl, pH 7.0, containing 10 mM lactose and 4 mM β-mercaptoethanol and applied to the column. Elution was performed with a linear gradient of 10 mM NaCl to 150 mM NaCl in 10 mM Tris-HCl, pH 7.0, containing 10 mM lactose and 4 mM β-mercaptoethanol at a 1 ml/min flow rate. Protein was determined by the method of Bradford (6).

Collection of washings from the skin was accomplished by immersing a female *X. laevis* in 75 ml of TEM in a plastic bag after injection into the dorsal lymph sac of 100 μg of L-epinephrine (Calbiochem-Behring Corp., La Jolla, CA) in 300 μl of 150 mM NaCl. The washings were applied directly to a 2-ml affinity column of lactose-conjugated agarose beads and lectin was purified by the same procedures used for the skin homogenate.

Electrophoresis and Amino Acid Analysis

Lectin preparations were evaluated by polyacrylamide slab gel electrophoresis

[1] *Abbreviations used in this paper:* TEM, 50 mM Tris-HCl, pH 7.6, plus 10 mM EDTA and 4 mM β-mercaptoethanol.

in SDS under reducing conditions as described previously (8) using a 15% sample gel. Protein bands were visualized with silver nitrate (18).

Amino acid analysis was performed at Scripps Clinic and Research Foundation, La Jolla, CA, on a Beckman 121 M amino acid analyzer following hydrolysis in 6 N HCl for 24 and 48 h at 100°C under reduced pressure. Methionine was estimated after 24 h. Cysteine was determined by performic acid oxidation of the sample prior to hydrolysis, and quantitation as cysteic acid.

Gel Filtration Chromatography. The native molecular weight of the purified lectin was determined by gel filtration chromatography in 10 mM Tris-HCl, 10 mM lactose, 100 mM NaCl, 4 mM β-mercaptoethanol, pH 6.8, using a Perkin-Elmer TSK-G-3000 column with a flow rate of 0.5 ml/min. Standards used were bovine serum albumin (66,000), carbonic anhydrase (29,000), cytochrome C (12,400), and aprotinin (6,500).

Isoelectric Focusing. Polyacrylamide slab gel isoelectric focusing was performed on a LKB horizontal slab gel apparatus using pre-poured 3.5 to 9.5 gels (LKB PAG plates, LKB Instruments, Inc., Rockville, MD). Gels were fixed with 10% aqueous trichloroacetic acid for 1 h, rinsed with distilled H_2O, and stained overnight with Coomassie Brilliant Blue G-250 (Sigma Chemical Co., St. Louis, MO) and destained in 10% acetic acid.

Antibody Preparation and Characterization

Antibody Production. Lectin used for immunization was purified by affinity chromatography followed by ion exchange chromatography to assure freedom from trace contaminants. Initial immunization of a rabbit was carried out by subcutaneous injection at multiple sites of 200 μg of lectin in complete Freund's adjuvant (Difco Laboratories, Detroit, MI). The rabbit was boosted with 25 μg of lectin in incomplete Freund's adjuvant beginning at 4 wk after initial injection and continuing at 2-wk intervals. A total of six boosts were given and serum was collected at 5 and 7 d after the final injection.

Affinity Purification of Antibody. Purification of the antibody for use in immunohistochemistry was done by immunoaffinity chromatography on a column containing 200 μg *X. laevis* skin lectin that had been coupled to 2 ml of cyanogen bromide–activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) according to the manufacturer's instructions. 2 ml of rabbit antiserum was recirculated through the column which was then extensively washed with 75 mM NaCl, 75 mM Na_2HPO_4/KH_2PO_4, pH 7.2 (PBS), and eluted with 0.2 M HCl adjusted to pH 2.2 with 2 M glycine. Fractions of 0.6 ml were collected directly into tubes containing 0.15 ml 1 M K_2HPO_4 and dialyzed against PBS overnight at 4°C. Bovine serum albumin was added to a final concentration of 0.1 mg/ml and the affinity-purified antibody was stored at 4°C in 0.02% sodium azide.

Immunoblotting. Antigens that react with the antiserum were identified by electrophoresis and immunoblotting procedures, using peroxidase-anti-peroxidase and 4-chloro-1-napthol to detect bound primary antibody, all as previously described (8).

Dot-Immunobinding Assay. Quantitation of *X. laevis* lectin in skin washings was performed by directly blotting twofold dilutions onto nitrocellulose sheets (Bio-Rad Laboratories, Richmond, CA) and reaction with antiserum, peroxidase-anti-peroxidase, and 4-chloro-1-napthol, all as described for immunoblotting.

Electron Microscope Immunohistochemistry

Tissue preparation. 2–3-mm thick slices of skin were fixed for 1 h at room temperature in freshly prepared 3% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Tissue was then rinsed in two changes of buffer for 10 min and then postfixed for 1 h at room temperature in 1% OSO_4 in 0.1 M sodium phosphate buffer, pH 7.4. Samples were then dehydrated in ethanol and embedded in Epon 812 by standard procedures. Thin sections were cut and picked up on carbon-parlodian–coated nickel grids for immunostaining.

Immunostaining. Colloidal gold with a mean diameter of 9 nm was purchased from Janssen Life Sciences Products (Piscataway, NJ). Goat anti-rabbit IgG (heavy chain– and light chain–specific) was purchased from Cooper-Biomedical Inc. (Malvern, PA). Colloidal gold–IgG complexes were prepared as described previously (8).

Grids containing thin sections of frog skin were immunostained as described previously (8). The grids were incubated with solutions containing 3 μg/ml affinity-purified antibody for 2 h at room temperature. Control grids were incubated with 10 μg/ml of preimmune IgG. After reaction with the colloidal gold goat anti-rabbit IgG complex, grids were stained with saturated aqueous uranyl acetate followed by lead citrate before examination with a Zeiss EM 10 electron microscope.

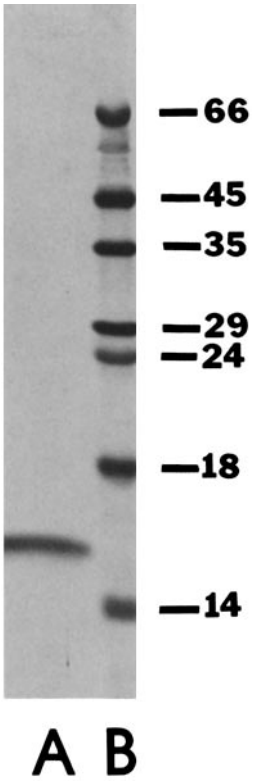

Figure 1. PAGE of lectin purified from *Xenopus laevis* skin extracts by affinity chromatography on lactose-conjugated agarose. The pure lectin (*A*) was eluted with lactose and electrophoresed on a polyacrylamide slab gel, as described in Materials and Methods, along with the indicated standards (*B*). The gel was stained with silver nitrate. The standards used were: lysozyme (14,300); β-lactoglobulin (18,400); trysinogen (24,000); carbonic anhydrase (29,000); pepsin (34,700); egg albumin (45,000); bovine albumin (66,000).

Results

Lectin Purification and Properties

Lectin was purified from extracts of *Xenopus laevis* skin by affinity chromatography on agarose gel beads conjugated with lactose. After extensive washing of the beads, pure lectin was eluted with lactose and migrated as a single band with an apparent molecular weight of ~16,000 upon SDS PAGE (Fig. 1). Upon gel filtration, the lectin migrated as a single peak with an apparent molecular weight of ~32,000. Like many related lectins (1) it is, therefore, a dimer. The skin of one adult female yielded from 1 to 1.5 mg of pure lectin.

In some preparations, no impurities were detectable even with a highly sensitive silver stain (Fig. 1). However, in several preparations, trace contaminants were detected, which could be removed by DEAE ion exchange chromatography. The purified lectin behaved as a single entity on ion exchange chromatography. Like soluble lactose-binding lectins from other vertebrates (7, 9, 17, 26), it is rich in aspartic and glutamic acid (Table I). Upon isoelectric focusing in a slab gel, it can be resolved into three prominent bands with approximate pI 6.2, 6.4, and 6.5.

The effect of a series of concentrations of several sugars on binding of pure lectin to the affinity column was determined to evaluate the specificity of its carbohydrate-binding site. Binding could be completely inhibited with 15 mM lactose, but concentrations as high as 300 mM melibiose and 300 mM galactose had no detectable inhibitory effect. Therefore this lectin is similar to many other vertebrate β-galactoside binding lectins (1), and differs markedly from lectins purified from *Xenopus* oocytes (24) or serum (25), both of which react well with α-galactosides such as melibiose. Removal of β-mercaptoethanol by dialysis of purified lectin against repeated

Table I. Amino Acid Composition (Residues/100 Residues) of X. laevis Skin Lectin with M_r ~ 16,000

Amino acid	Residues/100
Asp	14.5
Thr	5.3
Ser	6.5
Glu	10.0
Pro	5.0
Gly	7.0
Ala	4.2
Cys	0.9
Val	6.4
Met	1.8
Ileu	8.3
Leu	7.3
Tyr	1.7
Phe	5.3
His	3.4
Lys	6.6
Arg	5.8

changes of buffer without this reducing agent greatly inhibited the binding of the lectin to the affinity column.

Specificity of Antiserum

A rabbit antiserum was raised against lectin that had been purified by affinity chromatography followed by DEAE cellulose chromatography, to assure removal of all trace contaminants. Immunoblots of whole skin extracts that had been electrophoresed and transferred to nitrocellulose showed only a single band (Fig. 2*a*) that migrated exactly with the purified lectin. Preimmune serum gave no reaction on immunoblots (Fig. 2*b*).

Epinephrine-induced Lectin Secretion

It has been established that injection of epinephrine induces marked secretion of biologically active small peptides from *X. laevis* skin (14). Since we were interested in the possibility that skin lectin was also secreted, we collected cutaneous washings after injection either of epinephrine or of saline, as described in Materials and Methods. We estimated the amount of lectin in the washings by the dot-immunoblot procedure described in Materials and Methods, using serial twofold dilutions of the washings bound to nitrocellulose (Fig. 3). The washings from the epinephrine-injected animal gave a clear positive result out to dilutions of 1:128, whereas those from the saline-injected animal gave a faint positive only at 1:2 (Fig. 3). This indicates that there was massive secretion of lectin upon epinephrine injection.

We also analyzed the nature of the secreted material by staining nitrocellulose transfers prepared after PAGE. Amido black staining of the transfers showed several major bands, but the lectin was not one them (Fig. 4*a*). Immunoblotting showed a major band with an apparent molecular weight of ~16,000 which migrated exactly with purified lectin (Fig. 4*b*). We also detected a very faint antibody-binding band with an apparent molecular weight of ~32,000. It is possible that this is an undissociated dimeric form of the lectin. However, it is also possible that it represents yet another lectin which is immunologically cross-reactive with the ~16,000-mol-wt lectin, by analogy with recent findings in the rat (9). The faint 32,000-mol-wt band was never observed on immunoblots of whole skin, and would not be expected to make a major contribution to the total immunoreactive material observed in immunohistochemical studies.

In preliminary studies we found that cutaneous secretions collected without β-mercaptoethanol were much more viscous than those collected with this reducing agent. We wondered if the lectin was normally linked by disulfide bonds to other secreted materials. To test this we collected epinephrine-induced secretions without β-mercaptoethanol, and examined the released material by gel electrophoresis also done without mercaptoethanol. The lectin was located on immunoblots of a nitrocellulose transfer. All the lectin on these immunoblots had an apparent molecular weight of ~16,000 and, aside from faint staining of a 32-kD band, no other immunoreactive

Figure 2. Immunoblot of whole skin extract stained with (*A*) immune serum or (*B*) preimmune serum. Antibody binding was detected with peroxidase-conjugated second antibody and 4-chloro-1-napthol, as described in Materials and Methods.

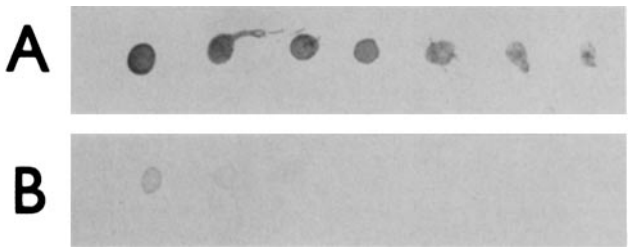

Figure 3. Dot immunoblots of serial twofold dilutions of cutaneous washings from epinephrine-injected (*A*) or saline-injected (*B*) *X. laevis*. The top row has dilutions from 1:2 to 1:128 from cutaneous washings of an epinephrine-treated frog, and the bottom similar dilutions from a saline-treated frog. There is a clear positive reaction in the top sample, even at 1:128, the highest dilution tested. Even the 1:2 dilution of the saline-injected sample is barely positive. Determination of antibody binding to the extracts was done with peroxidase-conjugated second antibody, as described in Materials and Methods.

bands were found. Therefore, the lectin is not linked by disulfide bands to either large glycoconjugates in the secretions or to toxic peptides of a size large enough to produce a detectable shift in apparent molecular weight of the 16,000-mol-wt band.

Because secretion of lectin with M_r ~16,000 was so prominent after epinephrine injection, we used skin washings as the starting material for purification of several preparations of lectin. We obtained ~2 mg of pure lectin from the washings of a single epinephrine-injected animal, more than could be obtained from skin homogenates. This is, therefore, the procedure of choice for obtaining crude lectin for purification. The smaller yield obtained from skin homogenates probably reflects both inefficient dissection and failure to completely disrupt the skin even with vigorous homogenization.

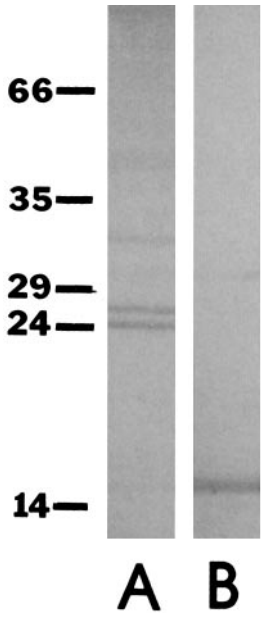

Figure 4. PAGE and immunoblotting of cutaneous washings from an epinephrine-injected *X. laevis*. The washings were electrophoresed on a slab gel and lanes were transferred to nitrocellulose and stained either with Amido black (*A*) or with antibody, peroxidase-conjugated second antibody, and 4-chloro-1-napthol (*B*). The dark band on the immunoblot migrated exactly with the purified lectin.

Immunohistochemical Studies. Frog skin contains two prominent glandular structures, granular glands, and mucous glands (Fig. 5). Upon epinephrine injection, the morphology of the granular glands is changed radically, reflecting an outpouring of the contents of this gland, including intact peptide-containing granules, as described by Dockray and Hopkins (14). Since the granular glands are so affected by epinephrine injection, which also releases so much lectin, we expected that lectin would be concentrated in these structures. This was confirmed by immunohistochemistry with the electron microscope, which showed substantial binding of affinity-purified antibody in the cytoplasm of an unstimulated granular gland cell (Fig. 6*a*). Reaction with antibody raised against the lectin was specific, since exposure of parallel sections to preimmune immunoglobulin gave no significant staining (Fig. 6*b*).

Secretion of lectin from the granular glands was also demonstrated directly by immunohistochemistry. In an animal injected with epinephrine, there was substantial lectin in the secreted material within the duct of the granular gland (Fig.

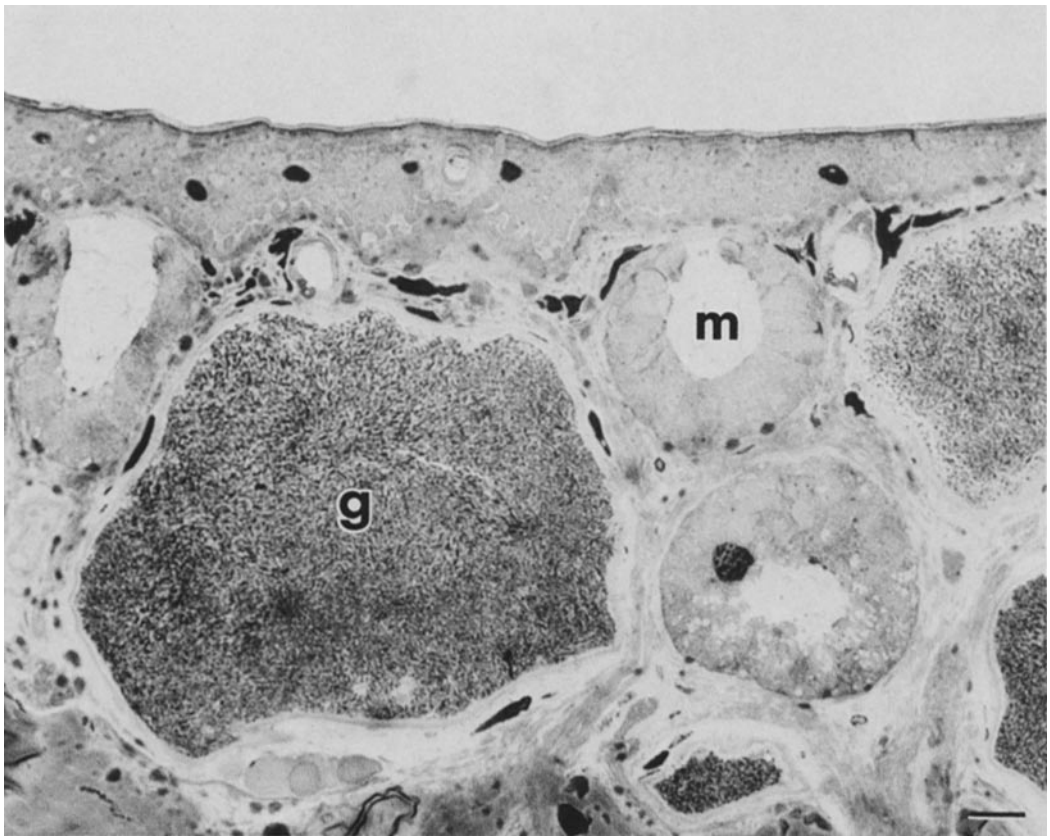

Figure 5. Glands in the skin of *X. laevis*. Skin was fixed and embedded by the procedures described in Materials and Methods for electron microscopy and sections were stained with hematoxylin and eosin and examined with a light microscope. The skin contains numerous granular glands (*g*) and mucous glands (*m*). Bar, 10 μm.

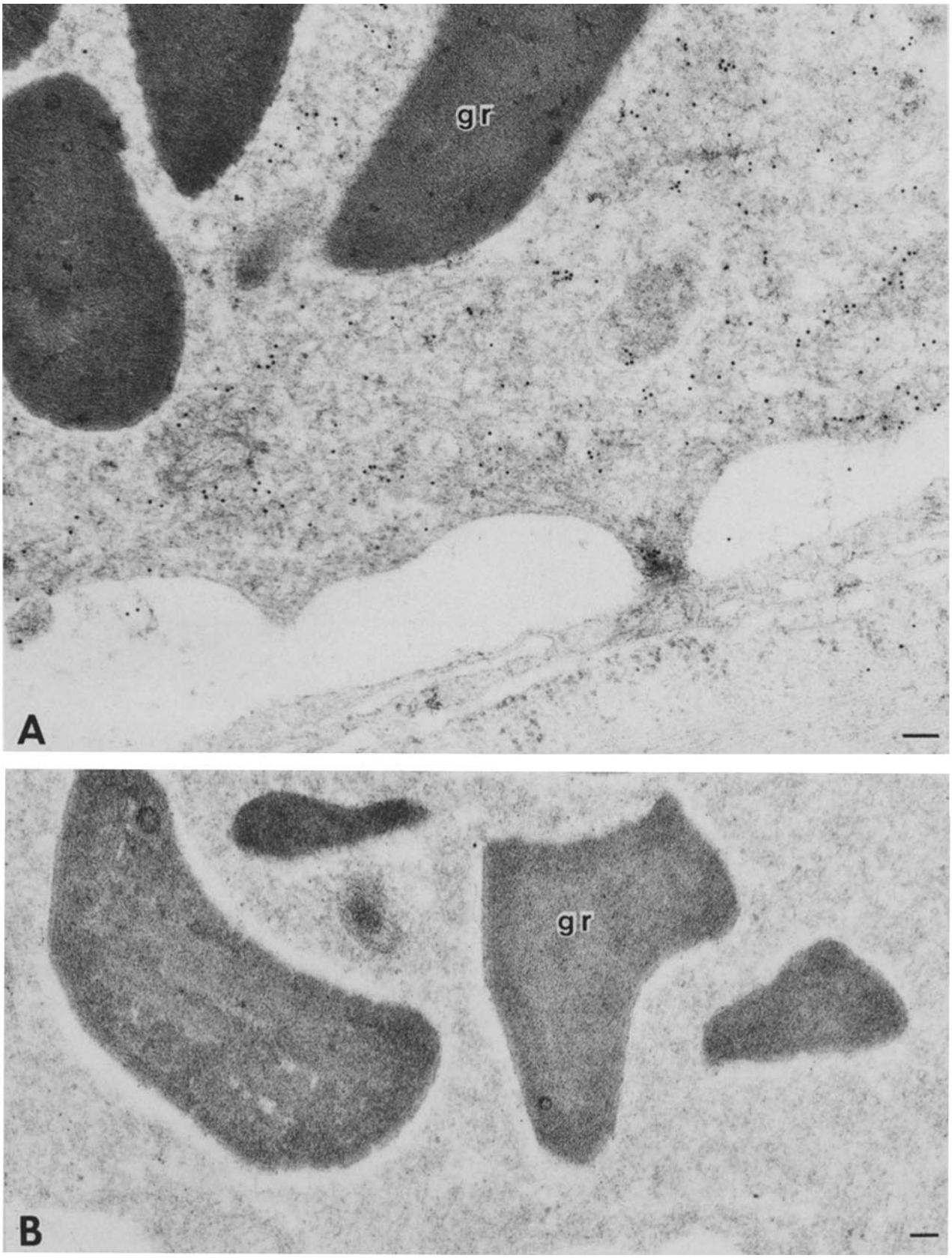

Figure 6. Immunohistochemical localization of lectin in the cytoplasm of a granular gland cell. Sections through a granular gland were reacted either with affinity-purified antibody raised against purified lectin (*A*) or with three times as much preimmune IgG (*B*). The sections were then stained with colloidal gold–conjugated goat anti-rabbit immunoglobulin. There is abundant lectin in the cytoplasm, but granules (*gr*) do not contain lectin. Bar, 0.1 μm.

7*a*). Again, staining was specific, since reaction with preimmune immunoglobulin gave no significant staining (Fig. 7*b*).

Lectin was also detected in the cytoplasm of mucous gland cells (Fig. 8). Globules of mucous in other regions of this cell contained no lectin (not shown). The morphology of the mucous gland showed no obvious change upon injection with epinephrine, so it is not known if cytoplasmic lectin is also secreted from mucous gland cells.

Discussion

The lectin that we purified from *Xenopus laevis* skin and its secretions closely resembles soluble β-galactoside-binding lec-

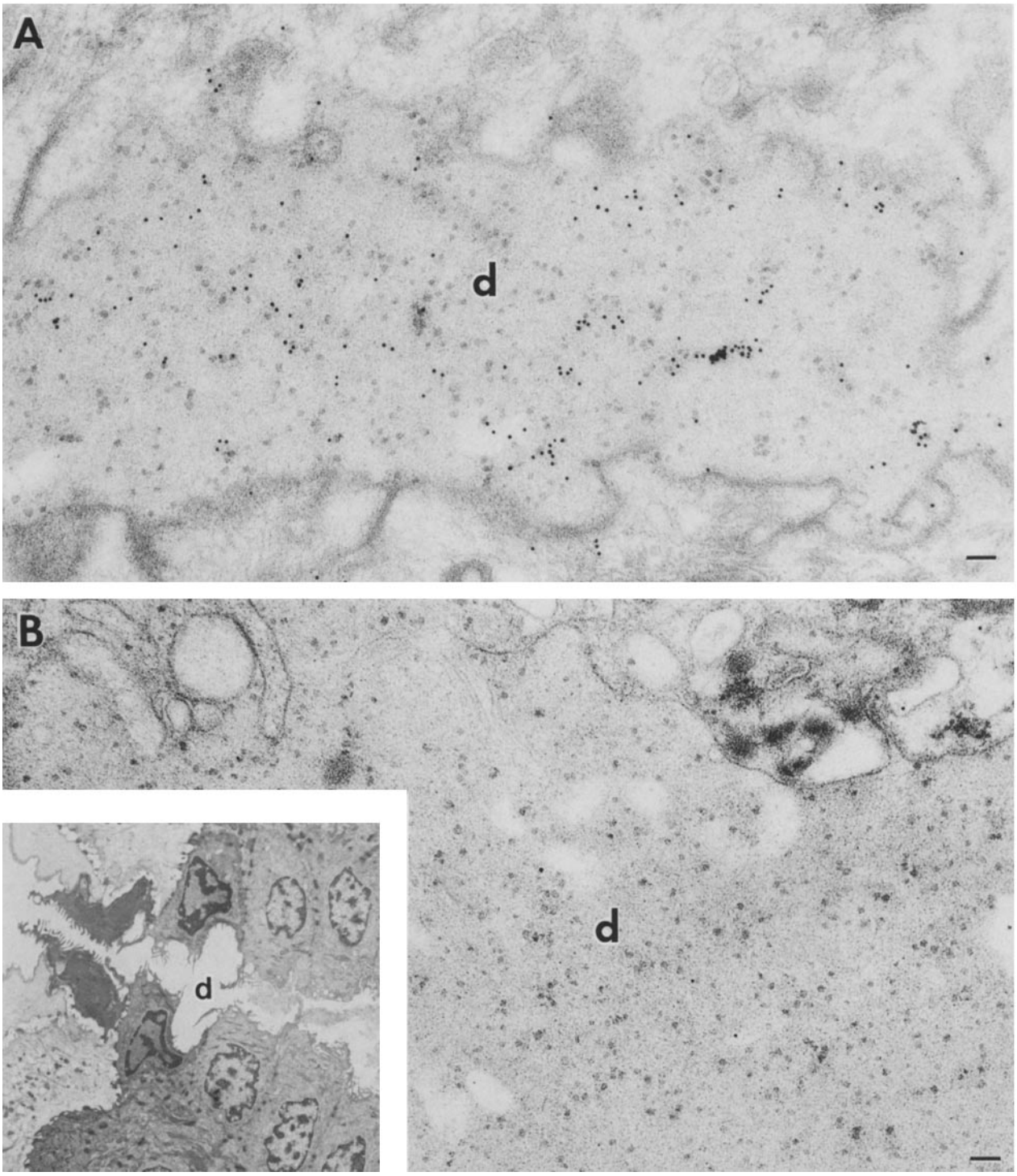

Figure 7. Immunohistochemical demonstration of lectin in the secreted material in the lumen of the granular gland duct of epinephrine-stimulated *Xenopus laevis*. Sections through the duct (*d*) of an epinephrine-stimulated granular gland were reacted with (*A*) affinity-purified anti-lectin or (*B*) preimmune IgG. The sections were then stained with colloidal gold–conjugated goat anti-rabbit IgG. (*Inset*) Low magnification electron micrograph of the lumen of the duct (*d*) of the granular gland, for orientation. Bars, 0.1 μm.

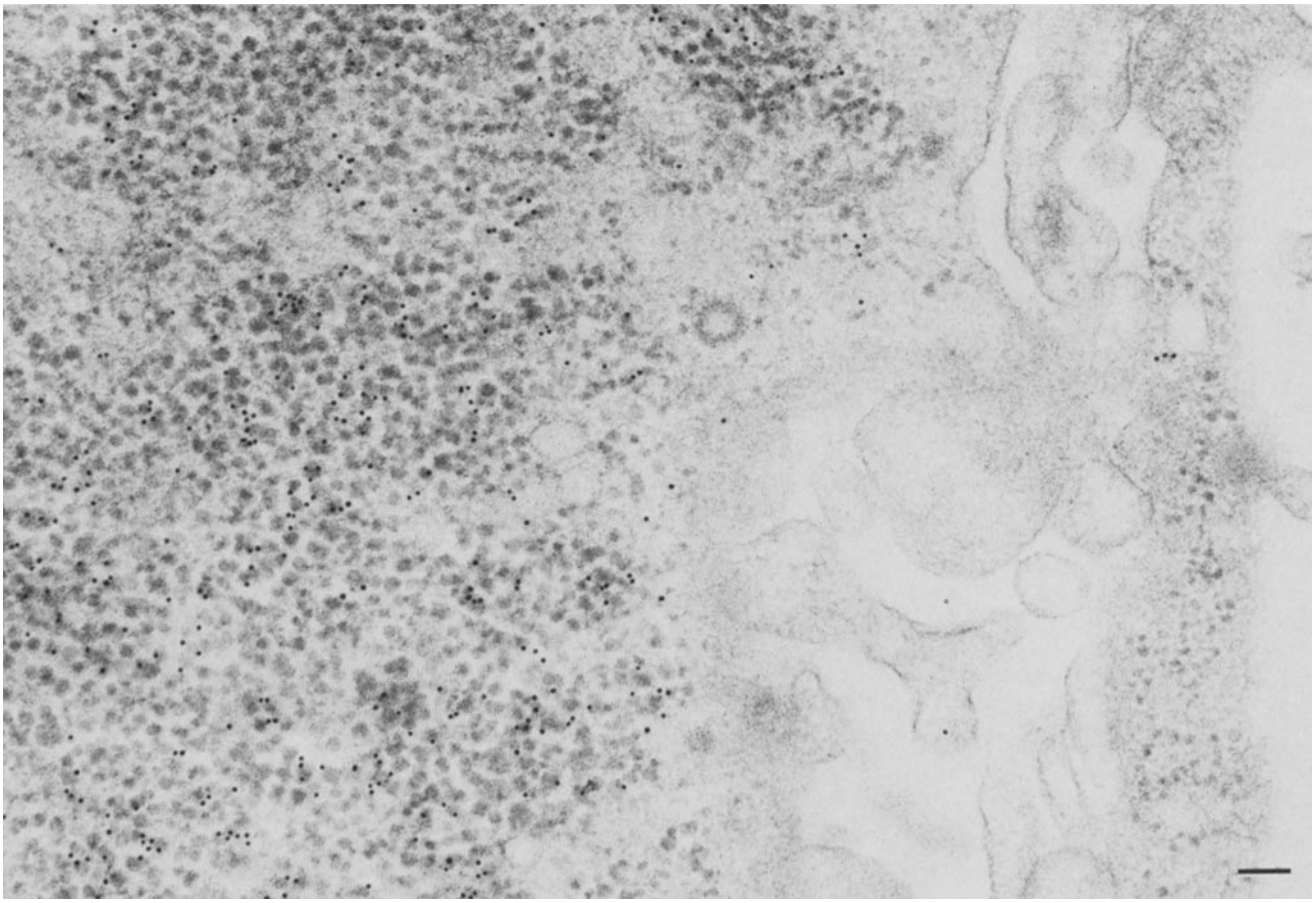

Figure 8. Immunohistochemical localization of lectin in the cytoplasm of a mucous gland cell. The section was reacted with affinity-purified anti-lectin followed by colloidal gold-labeled goat anti-rabbit IgG. Controls stained with preimmune serum were negative. Bar, 0.1 μm.

tins from other vertebrates, including eel (17), chicken (5, 13, 20), snake (16), and several mammals (7–10, 12, 23, 26). Like these, it does not require detergents for solubilization (which distinguishes it from lectins integrated in membranes [1]), has a subunit M_r in the range of 15,000, and binds lactose. It differs strikingly from two other soluble lectins already purified from *Xenopus laevis* (24, 25). The *Xenopus* lectin from oocytes (24) has two subunits with apparent molecular weights of ~43,000 and 45,000 and binds α-galactosides at least as well as β-galactosides, as does the lectin made in *Xenopus* liver and secreted into the serum (25), which has a subunit in the molecular weight range of ~69,000. Although the *Xenopus* skin lectin is probably closely related to a rat 14,500-mol-wt lectin, potent antisera raised against the rat lectin (9) did not cross-react with nitrocellulose transfers of the *Xenopus* skin lectin.

Previous studies have indicated that soluble β-galactoside-binding lectins from vertebrates may be secreted (1). As already noted, this was clearly shown in the case of chicken-lactose lectin II in chicken intestine (3). In this case, the lectin is packaged in secretory vesicles (3). However, evidence for secretion of other β-galactoside-binding soluble vertebrate lectins has been less direct. Chicken lactose lectin I is intracellular in immature muscle but is later concentrated extracellularly, suggesting secretion (2). Chicken lactose lectin I is also found extracellularly in pancreas (4). The ~14,500-mol-wt rat β-galactoside-binding lectin is found in the cytoplasm of several lung cell types, but its prominent extracellular location in elastic fibers (8) suggests that it is secreted there. In these instances secretion from the cytoplasm, by an unknown mechanism, has been inferred but not directly demonstrated. Given the general view that secretion proceeds by packaging in vesicles and not directly from the cytoplasm, it has been difficult to accept that cytoplasmic lectin is actually secreted.

The present experiments show secretion of a cytoplasmic (nonvesicular) β-galactoside-binding lectin from *Xenopus laevis* granular gland by both immunohistochemistry and by collection of copious lectin in the cutaneous washings of epinephrine-injected animals. This case is, of course, unusual because it involves massive secretion from granular gland cells by rupture of plasma membranes at the base of their ducts. This is induced by contraction of the surrounding myoepithelial cells which squeeze out cytoplasm and granules (14). The membranes then reseal so the cells can resynthesize these contents (14). Although such a specialized and dramatic mechanism would not appear to be responsible for the secretion of cytoplasmic lectins in all cases, these results raise the possibility that more subtle breaking and resealing of plasma membranes may be involved in the secretion of cytoplasmic lectins in other tissues, such as lung (8) and skeletal muscle (2). It is also possible that those cases do involve some massive cellular rupture like that seen here, or even destruction of some cells rich in the lectin and release of cytoplasmic contents.

The function of the lectin secreted by the granular glands

is not known. Indeed we cannot rule out the possibility that the function of the lectin is in the cytoplasm, and that it just happens to be released along with other cellular contents. The secretion is rich in a variety of biologically active peptides and amines (11, 14, 15), many of which are also found in mammalian gastrointestinal tract and brain (15), and which can act as toxins. Massive release of these compounds is believed to act as a defense against predators (11) who frequently drop frogs that have secreted their cutaneous toxins, and learn to avoid them. This raises the possibility that lectin in *Xenopus* skin may itself have a toxic effect, as may those found in snake venoms (16, 21). The lectin could also serve to organize glycoconjugates in cutaneous mucins, providing a meshwork to hold the toxin granules on the skin.

Another possibility is that it acts like the carbohydrate-binding subunits of the plant lectins ricin and abrin which combine with toxins (22). Association of the carbohydrate-binding sites of these lectins with glycoconjugates on the surface of cells serves to deliver the toxins to their targets. In the case of the plant lectins, toxin association is via disulfide bonds. These would be cleaved by β-mercaptoethanol present in our media and might, therefore, have escaped our notice. However in our studies of crude cutaneous secretions isolated and electrophoresed under conditions which would preserve disulfide bonds, we found no evidence for an increase in lectin molecular weight, which would be expected if it were coupled to toxin. Therefore, if lectin and toxin associate it is not by a disulfide bond.

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References

1. Barondes, S. H. 1984. Soluble lectins: a new class of extracellular proteins. *Science.* 223:1259–1264.

2. Barondes, S. H., and P. L. Haywood-Reid. 1981. Externalization of an endogenous chicken muscle lectin with in vivo development. *J. Cell Biol.* 91:568–572.

3. Beyer, E. C., and S. H. Barondes. 1982. Secretion of endogenous lectin by chicken intestinal goblet cells. *J. Cell Biol.* 92:28–33.

4. Beyer, E. C., K. Tokuyasu, and S. H. Barondes. 1979. Localization of an endogenous lectin in chicken liver, intestine, and pancreas. *J. Cell Biol.* 82:565–571.

5. Beyer, E. C., S. E. Zweig, and S. H. Barondes. 1980. Two lactose-binding lectins from chicken: purified lectin from intestine is different from those in liver and muscle. *J. Biol. Chem.* 255:4236–4239.

6. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.

7. Briles, E. B., W. Gregory, P. Fletcher, and S. Kornfeld. 1979. Vertebrate lectins. Comparison of properties of β-galactoside-binding lectins from tissues of calf and chicken. *J. Cell Biol.* 81:528–537.

8. Cerra, R. F., P. L. Haywood-Reid, and S. H. Barondes. 1984. Endogenous mammalian lectin localized extracellularly in lung elastic fibers. *J. Cell Biol.* 98:1580–1589.

9. Cerra, R. F., M. A. Gitt, and S. H. Barondes. 1985. Three soluble rat β-galactoside-binding lectins. *J. Biol. Chem.* 260:10474–10477.

10. Childs, R. A., and T. Feizi. 1979. β-galactoside-binding muscle lectins of man and monkey show antigenic cross-reactions with those of bovine origin. *Biochem. J.* 183:755–758.

11. Daly, J. W., and B. Witkop. 1971. Chemistry and Pharmacology of Frog Venoms. *In* Venomous Animals and Their Venoms. Vol. 2. W. Bücherl and E. Buckley, editors. Academic Press, Inc., New York. 497–519.

12. De Waard, A., S. Hickman, and S. Kornfeld. 1976. Isolation and properties of β-galactoside-binding lectins of calf heart and lung. *J. Biol. Chem.* 251:7581–7587.

13. Den, H., and D. A. Malinzak. 1977. Isolation and properties of β-D-galactoside-specific lectin from chick embryo thigh muscle. *J. Biol. Chem.* 252:5444–5448.

14. Dockray, G. J., and C. R. Hopkins. 1975. Caerulein secretion by dermal glands in *Xenopus laevis*. *J. Cell Biol.* 64:724–733.

15. Erspamer, V., and P. Melchiorri. 1980. Active polypeptides: from amphibian skin to gastrointestinal tract and brain of mammals. *Trends Pharmacol. Sci.* 1:391–395.

16. Gartner, T. K., and M. L. Ogilvie. 1984. Isolation and characterization of three Ca^{2+}-dependent β-galactoside-specific lectins from snake venoms. *Biochem. J.* 224:301–307.

17. Levi, G., and V. I. Teichberg. 1981. Isolation and physiochemical characterization of electrolectin, a β-D-galactoside-binding lectin from the electric organ of *Electrophorus electricus*. *J. Biol. Chem.* 256:5735–5740.

18. Morrissey, J. H. 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. *Anal. Biochem.* 117:307–310.

19. Nitta, K., G. Takayanagi, Y. Terasaki, and H. Kawauchi. 1984. Studies on three kinds of lectins from *Xenopus laevis* skin. *Experientia.* 40:712–713.

20. Nowak, T. P., D. Kobiler, L. Roel, and S. H. Barondes. 1977. Developmentally regulated lectin from embryonic chick pectoral muscle. Purification by affinity chromatography. *J. Biol. Chem.* 252:6026–6030.

21. Ogilvie, M. L., and T. K. Gartner. 1984. Identification of lectins in snake venoms. *J. Herpetol.* 18:285–290.

22. Olsnes, S., and A. Pihl. 1976. Abrin, ricin, and their associated agglutinins. *In* The Specificity and Action of Animal, Bacterial and Plant Toxins (Receptors and Recognition, series B, Vol. 1). P. Cuatrecasas, editor. Chapman and Hall, London. 131–173.

23. Powell, J. T., and P. L. Whitney. 1980. Postnatal development of rat lung. Changes in lung lectin, elastin, acetylcholinesterase and other enzymes. *Biochem. J.* 188:1–8.

24. Roberson, M. M., and S. H. Barondes. 1982. Lectin from embryos and oocytes of *Xenopus laevis*: purification and properties. *J. Biol. Chem.* 257:7520–7524.

25. Roberson, M. M., A. P. Wolffe, J. R. Tata, and S. H. Barondes. 1985. Galactoside-binding serum lectin of *Xenopus laevis*: estrogen dependent hepatocyte synthesis and relationship to oocyte lectin. *J. Biol. Chem.* 260:11027–11032.

26. Whitney, P., S. Maxwell, U. Ryan, and D. Massaro. 1985. Synthesis and binding of lactose-specific lectin by isolated lung cells. *Am. J. Physiol.* 248:C258–C264.